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Standard Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi[1]

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This standard has been approved for use by agencies of the Department of Defense. Consult the DoD Index of Specifications and Standards for the specific year of issue which has been adopted by the Department of Defense.

1. Scope

1.1 This practice covers determination of the effect of fungi on the properties of synthetic polymeric materials in the form of molded and fabricated articles, tubes, rods, sheets, and film materials. Changes in optical, mechanical, and electrical properties may be determined by the applicable ASTM methods.

1.2 The values stated in SI units are to be regarded as the standard.

2. Referenced Document

2.1 *ASTM Standard:*

D 618 Methods of Conditioning Plastics and Electrical Insulating Materials for Testing[2]

3. Summary of Practice

3.1 The procedure described herein consists of (*1*) selection of suitable specimens for determination of pertinent properties, (*2*) inoculation of the specimens with suitable organisms, (*3*) exposure of inoculated specimens under conditions favorable to growth, (*4*) examination and rating for visual growth, and (*5*) removal of the specimens and observations or testing, either before cleaning or after cleaning and reconditioning.

NOTE 1—Since the procedure involves handling and working with fungi, it is recommended that personnel trained in microbiology perform the portion of the procedure involving handling of organisms and inoculated specimens.

4. Significance and Use

4.1 The resin portion of these materials is usually fungus-resistant in that it does not serve as a carbon source for the growth of fungi. It is generally the other components, such as plasticizers, cellulosics, lubricants, stabilizers, and colorants, that are responsible for fungus attack on plastic materials. It is important to establish the resistance to microbial attack under conditions favorable for such attack, namely, a temperature of 2 to 38°C (35 to 100°F) and a relative humidity of 60 to 100 %.

4.2 The effects to be expected are as follows:

4.2.1 Surface attack, discoloration, loss of transmission (optical).

4.2.2 Removal of susceptible plasticizers, modifiers, and lubricants, resulting in increased modulus (stiffness), changes in weight, dimensions, and other physical properties, and deterioration of electrical properties such as insulation resistance, dielectric constant, power factor, and dielectric strength.

4.3 Often the changes in electrical properties are due principally to surface growth and its associated moisture and to pH changes caused by excreted metabolic products. Other effects include preferential growths caused by nonuniform dispersion of plasticizers, lubricants, and other processing additives. Attack on these materials often leaves ionized conducting paths. Pronounced physical changes are observed on products in film form or as coatings, where the ratio of surface to volume is high, and where nutrient materials such as plasticizers and lubricants continue to diffuse to the surface as they are utilized by the organisms.

4.4 Since attack by organisms involves a large element of chance due to local accelerations and inhibitions, the order of reproducibility may be rather low. To assure that estimates of behavior are not too optimistic, the greatest observed degree of deterioration should be reported.

4.5 Conditioning of the specimens, such as exposure to leaching, weathering, heat treatment, etc., may have significant effects on the resistance to fungi. Determination of these effects is not covered in this practice.

5. Apparatus

5.1 *Glassware*—Glass vessels are suitable for holding specimens when laid flat. Depending on the size of the specimens, the following are suggested:

5.1.1 For specimens up to 75 mm (3 in.) in diameter, 150-mm (6-in.) covered Petri dishes.

5.1.2 For 75 mm (3 in.) and larger specimens, such as tensile and stiffness strips, large Petri dishes, trays of borosilicate glass, or baking dishes up to 400 by 500 mm (16 by 20 in.) in size, covered with squares of window glass.

5.2 *Incubator*—Incubating equipment for all test methods shall maintain a temperature of 28 to 30°C (82.4 to 86°C) and a relative humidity not less than 85 %. Automatic recording of wet- and dry-bulb temperature is recommended.

6. Reagents and Materials

6.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specification are available.[3] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

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[1] This practice is under the jurisdiction of ASTM Committee G-3 on Durability of Nometallic Materials and is the direct responsibility of Subcommittee G03.04 on Biological Deterioration.

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[2] *Annual Book of ASTM Standards,* Vol 08.01.

6.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean distilled water or water of equal purity.

6.3 *Nutrient-Salts Agar*[4]—Prepare this medium by dissolving in 1 L of water the designated amounts of the following reagents:

Potassium dihydrogen orthophosphate (KH_2PO_4)	0.7 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.7 g
Ammonium nitrate (NH_4NO_3)	1.0 g
Sodium chloride (NaCl)	0.005 g
Ferrous sulfate ($FeSO_4 \cdot 7H_2O$)	0.002 g
Zinc sulfate ($ZnSO_4 \cdot 7H_2O$)	0.002 g
Manganous sulfate ($MnSO_4 \cdot H_2O$)	0.001 g
Agar	15.0 g
Potassium monohydrogen orthophosphate (K_2HPO_4)	0.7 g

Sterilize the test medium by autoclaving at 121°C (250°F) for 20 min. Adjust the pH of the medium by the addition of 0.01 *N* NaOH solution so that after sterilization the pH is between 6.0 and 6.5. Prepare sufficient medium for the required tests.

6.4 *Mixed Fungus Spore Suspension*:

NOTE 2—Since a number of other organisms may be of specific interest for certain final assemblies or components, such other pure cultures of organisms may be used if agreed upon by the purchaser and the manufacturer of the plastic. Reference (1) illustrates such a choice.

6.4.1 Use the following test fungi in preparing the cultures:

Fungi	ATCC No.[A]	MYCO No.[B]
Aspergillus niger	9642	386
Penicillium pinophilum[C]	11797	391
Chaetomium globosum	6205	459
Gliocladium virens	9645	365
Aureobasidium pullulans	15233	279c

[A] American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

[B] Mycological Services, Box 1056, Crawfordsville, IN 47933.

[C] Historically known as funiculosm.

Maintain cultures of these fungi separately on an appropriate medium such as potato dextrose agar. The stock cultures may be kept for not more than 4 months at approximately 3 to 10°C (37 to 50°F). Use subcultures incubated at 28 to 30°C (82 to 86°F) for 7 to 20 days in preparing the spore suspension.

6.4.2 Prepare a spore suspension of each of the five fungi by pouring into one subculture of each fungus a sterile 10-mL portion of water or of a sterile solution containing 0.05 g/L of a nontoxic wetting agent such as sodium dioctyl sulfosuccinate. Use a sterile platinum or nichrome inoculating wire to scrape gently the surface growth from the culture of the test organism.

6.4.3 Pour the spore charge into a sterile 125-mL glass-stoppered Erlenmeyer flask containing 45 mL of sterile water and 10 to 15 solid glass beads, 5 mm in diameter. Shake the flask vigorously to liberate the spores from the fruiting bodies and to break the spore clumps.

6.4.4 Filter the shaken suspension through a thin layer of sterile glass wool in a glass funnel into a sterile flask in order to remove mycelial fragments.

6.4.5 Centrifuge the filtered spore suspension aseptically, and discard the supernatant liquid. Resuspend the residue in 50 mL of sterile water and centrifuge.

6.4.6 Wash the spores obtained from each of the fungi in this manner three times. Dilute the final washed residue with sterile nutrient-salts solution (Note 3) in such a manner that the resultant spore suspension shall contain 1 000 000 ± 200 000 spores/mL as determined with a counting chamber.

6.4.7 Repeat this operation for each organism used in the test and blend equal volumes of the resultant spore suspension to obtain the final mixed spore suspension.

NOTE 3—Nutrient salts solution is identical with the composition for nutrient salts agar given in 6.3 except that the agar is omitted.

6.4.8 The spore suspension may be prepared fresh each day or may be held in the refrigerator at 3 to 10°C (37 to 50°F) for not more than 4 days.

7. Viability Control

7.1 With each daily group of tests place each of three pieces of sterilized filter paper, 25 mm (1 in.) square, on hardened nutrient-salts agar in separate Petri dishes. Inoculate these with the spore suspension by spraying the suspension from a sterilized atomizer[5] so that the entire surface is moistened with the spore suspension. Incubate these at 28 to 30°C (82 to 86°F) at a relative humidity not less than 85 % and examine them after 14 days' incubation. There shall be copious growth on all three of the filter paper control specimens. Absence of such growth requires repetition of the test.

8. Test Specimens

8.1 The simplest specimen may be a 50 by 50-mm (2 by 2-in.) piece, a 50-mm (2-in.) diameter piece, or a piece (rod or tubing) at least 76 mm (3 in.) long cut from the material to be tested. Completely fabricated parts or sections cut from fabricated parts may be used as test specimens. On such specimens, observation of effect is limited to appearance, density of growth, optical reflection or transmission, or manual evaluation of change in physical properties such as stiffness.

8.2 Film-forming materials such as coatings may be tested in the form of films at least 50 by 25 mm (2 by 1 in.) in size. Such films may be prepared by casting on glass and stripping after cure, or by impregnating (completely covering) filter paper or ignited glass fabric.

8.3 For visual evaluation, three specimens shall be inoculated. If the specimen is different on two sides, three specimens of each, face up and face down, shall be tested.

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[3] "Reagent Chemicals, American Chemical Society Specifications," Am. Chemical Soc., Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see "Reagent Chemicals and Standards," by Joseph Rosin, D. Van Nostrand Co., Inc., New York, NY, and the "United States Pharmacopeia."

[4] Agar and nutrient-salts agar are obtainable from biological laboratory supply sources.

[5] DeVilbiss No. 163 atomizer or equivalent has been found satisfactory for this purpose.

NOTE 4—In devising a test program intended to reveal quantitative changes occurring during and after fungal attack, an adequate number of specimens should be evaluated to establish a valid value for the original property. If five replicate specimens are required to establish a tensile strength of a film material, the same number of specimens shall be removed and tested for each exposure period. It is to be expected that values of physical properties at various stages of fungal attack will be variable; the values indicating the greatest degradation are the most significant (see 4.4). The *ASTM Manual on Presentation of Data and Control Chart Analysis STP 15D,* may be used as a guide.

9. Procedure

9.1 *Inoculation*—Pour sufficient nutrient-salts agar into suitable sterile dishes (see 5.1) to provide a solidified agar layer from 3 to 6 mm (⅛ to ¼ in.) in depth. After the agar is solidified, place the specimens on the surface of the agar. Inoculate the surface, including the surface of the test specimens, with the composite spore suspension by spraying the suspension from a sterilized atomizer[5] with 110 kPa (16 psi) of air pressure so that the entire surface is moistened with the spore suspension.

9.2 *Incubation*—Cover the inoculated test specimens and incubate at 28 to 30°C (82 to 86°F) and not less than 85 % relative humidity for a minimum of 21 days, recording the growth each week.

NOTE 5—Covered dishes containing nutrient agar are considered to have the desired humidity. Covers on large dishes may be sealed with masking tape.

9.3 *Observation for Visible Effects*—If the test is for visible effects only, remove the three specimens from the incubator and judge them as follows:

Observed Growth on Specimens	Rating
None	0
Traces of growth (less than 10 %)	1
Light growth (10 to 30 %)	2
Medium growth (30 to 60 %)	3
Heavy growth (60 % to complete coverage)	4

NOTE 6—Traces of growth may be defined as scattered, sparse fungus growth such as might develop from (*1*) a mass of spores in the original inoculum, or (*2*) extraneous contamination such as fingermarks, insect feces, etc. Continous cobwebby growth extending over the entire specimen, even though not obscuring the specimen, should be rated as 2.

NOTE 7—Considerable physical change in plastics may occur without much visual growth, hence some measure of change in physical property selected from those cited in the Appendix is recommended.

9.4 *Effect on Physical, Optical, or Electrical Properties*—Wash the specimens free of growth, immerse in an aqueous solution of mercuric chloride (1+1000) for 5 min, rinse in tap water, air dry overnight at room temperature, and recondition at the standard laboratory conditions defined in Methods D 618, 23 ± 1°C (73 ± 2°F) and 50 ± 2 % relative humidity, and test according to the respective methods used on control specimens (see the Appendix).

NOTE 8—For certain electrical tests, such as insulation resistance and arc resistance, specimens may be tested in the unwashed, humidified condition. Test values will be affected by surface growth and its associated moisture.

10. Report

10.1 The report shall include the following:

10.1.1 Organisms or organism used,

10.1.2 Time of incubation (if progressive),

10.1.3 Visual rating of fungus growth according to 9.3, and

10.1.4 Tabulation of progressive change in physical, optical, or electrical property against time of incubation. Give the number of observations, the mean, and the maximum observed change.

REFERENCES

(1) Bagdon, V. J., Military Specification Mil-P-43018(CE), "Plastic Sheets: Polyethylene Terephthalate, Drafting, Coated," June 13, 1961.

(2) Baskin, A. D., and Kaplan, A. M., "Mildew Resistance of Vinyl-Coated Fabrics," *Applied Microbiology*, APMBA, Vol 4, No. 6, November 1956.

(3) Berk, S., "Effect of Fungus Growth on Plasticized Polyvinyl Chloride Films," *ASTM Bulletin*, ASTBA, No. 168, September 1950, p. 53 (TP 181).

(4) Berk, S., Ebert, H., and Teitell, L., "Utilization of Plasticizers and Related Organic Compounds by Fungi," *Industrial and Engineering Chemistry*, IECHA, Vol 49, No. 7, July 1957, pp. 1115–1124.

(5) Brown, A. E., "Problem of Fungal Growth on Synthetic Resins, Plastics, and Plasticizers," *Modern Plastics*, MOPLA, Vol 23, 1946, p. 189.

(6) Ross, S. H., "Biocides for a Strippable Vinyl Plastic Barrier Material," Report PB-151-119, U. S. Department of Commerce, Office of Technical Services.

APPENDIX

(Nonmandatory Information)

X1. TEST METHODS FOR EVALUATION OF EFFECT OF FUNGI ON SYNTHETIC POLYMERIC MATERIALS

X1.1 For evaluation of the effect of fungi on mechanical, optical, and electrical properties, the following ASTM and other test methods are recommended.

Property	Methods
Tensile strength	D 638, D 882, D 1708[A]
Stiffness	D 747[A] TAPPI Test Method T 451-M-45 Fed. Std. No 191, Method 5204 (Clark Stiffness Test) Fed. Std. No 191, Method 5206 (Cantilever Bend Method)
Hardness	D 785[A]
Optical transmission	E 308[A]
Haze	D 1003[A]
Water vapor transmission	E 96[A]
Dielectric strength	D 149[A]
Dielectric constant-power factor	D 150[A]
Insulation resistance	D 257[A]
Arc resistance	D 495[A]

[A] These designations refer to the following ASTM methods:
D 149, Test Method for Dielectric Breakdown Voltage and Dielectric Strength of Electrical Insulating Materials at Commercial Power Frequencies[2]
D 150, Test Methods for A-C Loss Characteristics and Dielectric Constant (Permittivity) of Solid Electrical Insulating Materials[2]
D 257, Test Methods for D-C Resistance or Conductance of Insulating Materials[2]
D 495, Test Method for High-Voltage, Low-Current Arc Resistance of Solid Electrical Insulating Materials[2]
D 638, Test Method for Tensile Properties of Plastics[2]
D 747, Test Method for Stiffness of Plastics by Means of a Cantilever Beam[2]
D 785, Test Method for Rockwell Hardness of Plastics and Electrical Insulating Materials[2]
D 1003, Test Method for Haze and Luminous Transmittance of Transparent Plastics[2]
D 1708, Test Method for Tensile Properties of Plastics by Use of Microtensile Specimens[6]
E 96, Test Methods for Water Vapor Transmission of Materials in Sheet Form[7]
E 308, Rec. Practice for Spectrophotometry and Description of Color in CIE 1931 System[8]

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[6] *Annual Book of ASTM Standards*, Vol 08.02.

[7] *Annual Book of ASTM Standards*, Vols 08.03 and 15.09.

[8] *Annual Book of ASTM Standards*, Vol 06.01.

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